

INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK
WAGENINGEN, NEDERLAND
DIRECTEUR: Dr. J. G. TEN HOUTEN

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**SOME CONSIDERATIONS ON THE TRANSMISSION
OF NON-PERSISTENT VIRUSES BY APHIDS**

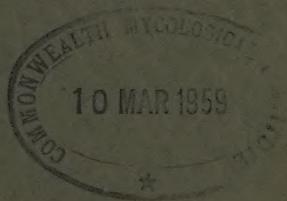
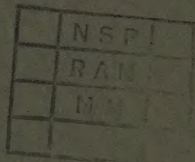
DOOR

H. A. VAN HOOF



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INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK (I.P.O.)

Office and main laboratory:

Binnenhaven 4a, tel. 2151, 2152 en 3641
Wageningen, The Netherlands.

Staff:

Director:

Dr. J. G. TEN HOUTEN.

Head of the Entomological Dept.:

Dr. H. J. DE FLUITER, Wageningen.

Deputy head of the Mycological Dept.:

Ir. A. M. VAN DOORN, Wageningen.

Head of the Nematological Dept.:

Dr. Ir. J. W. SEINHORST, Wageningen.

Head of the Plant Disease Resistance Dept.:

Dr. J. C. s'JACOB, Wageningen.

Head of the Virological Dept.:

Miss Dra. F. QUAK, Wageningen.

Deputy head of the Dept. for economic use of pesticides and aerial spraying in agriculture:

Miss M. C. KERSEN, Wageningen.

Research workers at the Wageningen Laboratory:

Ir. A. B. R. BEEMSTER, Virologist

Ir. J. A. DE BOKX, Virologist

Dr. Ir. L. BOS, Virologist

Ir. A. M. VAN DOORN, Phytopathologist

Dr. H. H. EVENHUIS, Entomologist

Dr. H. J. DE FLUITER, Entomologist

Dr. C. J. H. FRANSSEN, Entomologist

Dr. J. GROSIJEAN, Phytopathologist

Ir. N. HUBBELING, Phytopathologist and plantbreeder

Dr. J. C. s'JACOB, Phytopathologist and plantbreeder

Miss Dr. C. H. KLINKENBERG, Nematologist

Ir. R. E. LABRUYÈRE, Phytopathologist

Drs. H. P. MAAS GEESTERANUS, Phytopathologist

Dr. J. C. MOOI, Phytopathologist

Ir. H. DEN OUDEN, Nematologist

Miss Dra. H. J. PFAELTZER, Virologist

Miss Dra. F. QUAK, Virologist

Dr. Ir. J. W. SEINHORST, Nematologist

Ir. J. VAN DER SPEK, Phytopathologist

Ir. F. H. F. G. SPIERINGS, Plantphysiologist

Dr. F. TJALLINGII, Phytopathologist

Dr. J. H. VENKAMP, Biochemist

Drs. J. C. ZADOKS, Phytopathologist

Research workers elsewhere:

Ir. H. A. VAN HOOFF, Phytopathologist } detached to „Proefstation voor de Groenteteelt

Drs. L. E. VAN 't SANT, Entomologist } in de volle grond", Alkmaar, tel. K 2200-4568.

Drs. D. J. DE JONG, Entomologist } detached to „Proefstation voor de Fruitteelt in de

Ir. G. S. ROOSJE, Phytopathologist } volle grond", Wilhelminadorp, Goes, tel. K 1100-2261

Ir. T. W. LEFFERING, Phytopathologist/Virologist, detached to „Proeftuin Noord Limburg"
Venlo, tel. K 4700-2503.

Drs. G. SCHOLTEN, Phytopathologist, detached to „Proefstation voor de bloemisterij in Nederland", Aalsmeer, tel. K 2977-688.

Ir. G. P. TERMOHLEN, Phytopathologist, detached to „Proeftuin voor de Groente- en Fruitteelt onder glas", Naaldwijk, tel. K 1740-4545.

Guest workers:

Dr. P. A. VAN DER LAAN, Entomologist, „Laboratorium voor toegepaste Entomologie der Gemeente Universiteit", Amsterdam, tel. K 2900-56282.

Dr. Ir. G. S. VAN MARLE, Entomologist, Diepenveenseweg 226, Deventer, tel. K 6700-3617.

Ir. G. W. ANKERSMIT, Entomologist, „Laboratorium voor Entomologie", Agricultural University, Wageningen, tel. K 8370-2438.

Dr. Ir. J. B. M. VAN DINTHER, Entomologist, „Laboratorium voor Entomologie", Agricultural University, Wageningen, tel. K 8370-2438.

Aphidological Adviser:

Mr. D. HILLE RIS LAMBERS, Entomologist, T.N.O., Bennekom, tel. K 8379-458.

SOME CONSIDERATIONS ON THE TRANSMISSION OF NON-PERSISTENT VIRUSES BY APHIDS

H. A. VAN HOOF

Institute of Phytopathological Research, Wageningen, The Netherlands

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H. A. VAN HOOFF

Institute of Phytopathological Research, Wageningen, The Netherlands

INTRODUCTION

Studying the literature on the transmission of non-persistent viruses it appears that two hypotheses regarding its explanation exist. According to one of these, virus is taken up by the aphid together with the food. In the case of non-persistent viruses only virus present in the utmost tip of the stylets can be transmitted, because regurgitation is supposed to be impossible and inactivation of virus occurs in the aphid. The other hypothesis holds the idea that the virus is not carried inside the stylet-tip but on its outside.

The first hypothesis is based on the fact that the aphid does not suck actively but that the food is forced into the stylets by the pressure existing in the phloem (KENNEDY & MITTLER, 1953). The question arose how far variation in leaf turgor affects the uptake of virus by the aphid. We investigated this in various experiments.

METHODS AND RESULTS

Leaves of broad bean (*Vicia faba*) plants infected with lupin mosaic virus were frozen in a deep-freeze cabinet. After thawing the uptake of virus by *Myzus persicae* was studied but none of the aphids used was able to transmit the virus. However, mechanical transmissions with the aid of carborundum revealed the presence of active virus in the treated leaves.

Broad bean leaves infected with lupin mosaic virus and leaves of *Nicotiana glutinosa* infected with cabbage black ring spot virus were killed by keeping them for 30 minutes in water of 52°C. The thermal inactivation point of lupin mosaic virus lies between 60° and 70°C (MASTENBROEK, 1942), and that of cabbage black ring spot virus around 57°C (BEEMSTER, 1957). The leaves had not completely lost their turgor as those which had been subjected to freezing. Nevertheless, aphids were unable to pick up virus from these leaves, although it was found that mechanically transmissible virus was still present.

We tried to find out at what temperature the leaf was changed to such an extent that aphids could no longer transmit virus from them. For this purpose leaves were dipped for five minutes into water of 40°, 42° and so on up to 50°C, respectively. None of these treatments had any effect on the turgor of the leaves but leaves which had been subjected to water of 46°C and upward and which were kept on moistened filter paper in petri dishes, started soon to rot and disintegrated completely. We observed a decrease in the transmissibility of virus by aphids from leaves exposed to 46°C and more.

In another series of experiments the turgor of broad bean leaves infected with lupin mosaic virus was eliminated by dipping the leaves in different chemicals, viz. ether, carbon-tetrachloride, acetone, formaldehyde 40% and ethanol 96%. Aphids could only transmit virus from leaves dipped in ethanol, although less frequently than from untreated leaves. Dipping the leaf in ethanol did not immediately kill the leaf, probably as a consequence of the fact that the air had not completely been

removed from the intercellular spaces. Therefore we impregnated the leaves with ethanol *in vacuo*. Now all the intercellular spaces were filled with ethanol and the aphids could no longer transmit virus from these leaves. However, it was possible to infect healthy plants with sap obtained from these leaves.

Another way of reducing cell turgor was to keep infected broad bean leaves for 24 hours at different relative humidities. From dried leaves aphids transmitted virus with much more difficulty than from untreated leaves or even not at all. However, virus transmission proceeded again normally after restoring the turgor by floating the leaves on water.

Finally, plasmolysis was applied to impair cell turgor by means of a 20 % glucose solution. It was found that this procedure had no harmful effect on virus transmission by aphids.

DISCUSSION

According to the experiments with plasmolysed leaves cell turgor seems not to be essential for the transmission of non-persistent viruses by aphids. However, when the tissue of the host is killed by means of freezing, hot water or chemicals, the aphid is no longer able to transmit virus from the treated leaves. From virus-infected leaves, dipped for a short time in hot water, virus transmission was possible but to a lesser degree than from untreated leaves. The experiments with leaves kept at different relative humidities indicate that the water content of the leaf is an important factor in the transmission of virus by aphids.

Acquisition of virus is not directly correlated with the uptake of food. We observed aphids form their salivary sheaths mainly in the cell walls, that is in the middle lamellae. The stylets protrude by an up- and downward movement while saliva is secreted. If the moving stylets pierce the end of the secreted salivary sheath, there would be a direct contact between stylets and cell wall. Any influence of the water content of the host tissue, and especially of the cell wall, would not be expected in this case, if the virus could be transmitted directly behind the ridges of the mandibular or maxillary stylets (VAN HOOFF, 1957). It is more likely that virus diffused through the salivary sheath into the lumen of the sheath can be transported behind these ridges.

It is difficult to understand that virus can not be picked up by aphids from dead tissue. An explanation might be that the infective agent occurs in the host tissue in two forms, viz. as nucleic acid and as complete virus, and that the aphid transmits only the first form. Enzymes, liberated at the death of the cell, might inactivate the nucleic acid much more quickly than the complete virus particle.

SUMMARY

Non-persistent viruses are transmitted by aphids only in the tip of the stylets. However, it is uncertain whether virus is carried on the inner side or on the outer side of the stylet tip. Several authors are of the opinion that the uptake of virus by the aphids is connected with the uptake of food. The uptake of food is a process, for which pressure in the host is necessary. To trace the influence of pressure of the host tissue on the uptake of virus some trials were performed.

REFERENCES

- BEEMSTER, A. B. R., - 1957. Onderzoekingen over een virusziekte bij stoppelknollen (*Brassica rapa* var. *rapifera*). T. Pl. ziekten 63: 1-12.

- HOOF, H. A. VAN, - 1957. On the mechanism of transmission of some plant viruses. Proc. Kon. Akad. Wetensch. Ser. C. 60: 314-317.
- KENNEDY, J. S. & T. E. MITTLER, - 1953. A method of obtaining phloem sap via the mouth parts of aphids. Nature 171: 528.
- MASTENBROEK, C., - 1942. Enkele veldwaarnemingen over virusziekten van lupine en een onderzoek over haar mozaiekziekte. T. Pl. ziekten 48: 97-118.

DISCUSSION

VAN SLOGTEREN SR: Did Mr VAN HOOF consider the possibility that dipping the leaves in different chemicals might deter the aphids from penetrating these leaves?

VAN HOOF: Using a KCN-solution to impregnate the leaves we observed that the aphids pierced the epidermis in the same way as in untreated leaves and the aphids transmitted the virus very well. Now leaves dipped in KCN-solution do not change in external appearance. But when the leaf turgor has disappeared it takes much longer before the aphids start to puncture the epidermis after they have been placed on the leaves than in case of normal turgor. Besides this we did not see any difference in the behaviour of the aphids.

CHESSIN: Would the speaker care to comment on the difference between virus transmission by aphids from leaves with turgor reduced by plasmolysis as compared with turgor reduced by lower relative humidity?

VAN HOOF: It may be that by lowering the air humidity not only the cell content but also the cell wall which the aphids pierce, dries. By plasmolyzing the leaves in solutions, the water content of the cellwall is probably not changed, in contrast to that of the protoplasm.

MARTINI: Does Mr VAN HOOF think that uptake of food is necessary for virus transmission?

VAN HOOF: No, I do not think so. KENNEDY & MITTLER (Nature 171: 528, 1953) found that food is pressed into the aphid by phloem pressure. So when you eliminate the pressure in the leaf by plasmolysis the aphids cannot take up food.

MOERICKE: Although the pressure in the plant is of great importance for the uptake of food, the aphids are able to suck liquids which are not under pressure. I let *Myzus persicae* pierce through collodion membranes into red-coloured water and found that some of the aphids got a red-coloured digestive tract. Is not it possible that virus, besides being attached to the rough outer surface of the mandibles, is carried in the hole at the tip of the mouth parts where food and salivary canals meet, and that this virus is pressed by the saliva into the plant when the aphid punctures again?

VAN HOOF: It is a known fact that aphids can infect subsequent plants in short feeding periods. This would be less likely to occur when virus was carried only in the hole inside the insects mouth parts because this hole would be cleaned from virus in the first feeding after the uptake of virus and besides the first test plants no others would be infected. Therefore it is more likely that the virus is carried in the ridges on the outer surface of the mandibles from where it may be removed during puncturing of leaves by some process of exchange.

H. P. HANSEN: I should like to ask Mr VAN HOOF if he has tried the effect of plasmolyzing with salts instead of with sugar. I ask this because in mechanical inoculations the addition of sugar increases considerably the infectivity of the inoculum. Sugar may have some effect in virus transmission by aphids as well.

VAN HOOF: No I have not tried salts. But from leaves which have been deep-frozen aphids were not able to pick up virus. It may be that the deep-freezing process inactivates the virus or its components.

THOMSON: Has Mr VAN HOOF tried inhibitors on cabbage black ringspot virus e.g. formaldehyde?

VAN HOOF: No, I have not.

Mededelingen van het Instituut voor Plantenziektenkundig Onderzoek

Contributions of the Institute for Phytopathological Research

Binnenhaven 4a, Wageningen, The Netherlands

- No 137. KLINKENBERG, C. H. en J. W. SEINHORST, De nematicide werking van Na N-methyl dithiocarbamaat (Vapam) bij toepassing in de herfst. (The nematocidal properties of Na N-methyl-dithiocarbamate (Vapam) when applied in autumn). Meded. L.H.S. Gent, XXI: 397-400, 1956. Prijs no 137 en 138 samen f 0,40.
- No 138. SEINHORST, J. W., J. D. BULLO en C. H. KLINKENBERG, Een vergelijking van de nematicide werking van DD en van 3-5-dimethyltetrahydro-1-3-5-2H-thiadiazine-2-thion. (A comparison of the nematocidal properties of DD and of 3-5-dimethyltetrahydro-1-3-5-2H-thiadiazine-2-thione). Meded. L.H.S. Gent, XXI: 387-395, 1956. Prijs no 137 en 138 samen f 0,40.
- No 139. HOOFF, H. A. VAN en Sr. TOLSMAN, Virusziekte bij rabarber. (Virus disease of rhubarb). Meded. Dir. Tuinb., 19: 761-764, 1956. Prijs no 139 en 140 samen f 0,60.
- No 140. HOOFF, H. A. VAN, Verschil in reactie van wilde sla ten opzichte van besmetting met het slamozaiekvirus. (Differences in reaction of wild lettuces with regard to infection by Lactuca virus 1). Tijdschr. o. Plantenz., 62: 285-290, 1956. Prijs no 139 en 140 samen f 0,60.
- No 141. BEEMSTER, A. B. R., Onderzoekingen over een virusziekte bij stoppelknollen (Brassica rapa var. rapifera). (Investigations on a virus disease of turnip). Tijdschr. o. Plantenz., 63: 1-12, 1957. Prijs f 0,40.
- No 142. FRANSSEN, C. J. H., De levenswijze en de bestrijding van de tuinboonkever, (The biology and control of Bruchus rufimanus Boh.). Versl. Landbouwk. Onderz., No. 62. 10, 75 pp., 1956. Prijs f 2,75.
- No 143. FRANSSEN, C. J. H., De levenswijze en de bestrijding van de bonekever (Acanthoscelides obtectus Say). (Biology and control of the common bean weevil). Meded. Dir. Tuinb., 19: 797-809, 1956. Prijs f 0,55.
- No 144. NUVELDT, W. C., Levenswijze en bestrijding van de aspergevlug. (Platyparea poeciloptera Schrank) in Nederland. (Biology and control of Platyparea poeciloptera Schrank in the Netherlands). Versl. Landbouwk. Onderz., No 63. 4, 40 pp., 1956. Prijs f 1,65.
- No 145. VAUGHAN, EDWARD K., A device for the rapid removal of tannins from virus infected plant tissues before extraction of inoculum. Tijdschr. o. Plantenz., 266-270, 1956. Prijs no 145 en 146 samen f 0,30.
- No 146. VAUGHAN, EDWARD K., Attempts to transfer Rubus and Fragaria viruses into herbaceous hosts. Tijdschr. o. Plantenz., 62: 271-273, 1956. Prijs no 145 en 146 samen f 0,30.
- No 147. ZEYLLSTRA, H. H., Papierchromatografie als middel voor de diagnose van de ringvlekkenziekte van zoete kers. Een voorlopige mededeling. (Paper chromatography and diagnosis of ring spot disease in sweet cherry. A preliminary report). Tijdschr. o. Plantenz., 62: 325-326, 1956. Prijs no 147 en 148 samen f 0,25.
- No 148. QUAK, FRED.A., Meristeemcultuur, gecombineerd met warmtebehandeling, voor het verkrijgen van virusvrije anjerplanten. (Meristem culture, combined with heat treatment, in order to obtain virus-free carnation plants). Tijdschr. o. Plantenz., 63: 13-14, 1957. Prijs no 147 en 148 samen f 0,25.
- No 149. FRANSSEN, J. J., De landbouwluchtvaart in Nederland. (Agricultural aviation in the Netherlands). Landbouwwoorl., 13: 578-585, 1956. f 0,45.
- No 150. HUBBELING, N., New aspects of breeding for disease resistance in beans. Euphytica, 6: 111-141, 1957. Prijs no 150 f 3,-.
- No 151. QUAK, FRED.A., Bladvlekkenziekte bij spruitkool veroorzaakt door Mycosphaerella brassicicola (Fr.) Lindau. (Ringspot disease of brussels sprouts caused by Mycosphaerella brassicicola (Fr.) Lindau) Meded. Dir. Tuinb., 20: 317-320, 1957. Prijs no 151, 152 en 153 samen f 0,95.
- No 152. LABRUYÈRE, R. E., Enkele waarnemingen over de schimmel Elsinoë veneta (Burkh.) Jenk., de perfecte vorm van Sphaceloma necator (Ell. et Ev.) Jenk. en Shear. (Observations on Elsinoë veneta (Burkh.) the perfect form of Sphaceloma necator (Ell. et Ev.) on raspberry) Tijdschr. o. Plantenz., 63: 153-158, 1957. Prijs no 151, 152 en 153 samen f 0,95.

- No 176. s'JACOB, J. C., Phytopathologische Probleme bei der Resistenzzüchtung. Z. Pfl.-zücht., 40: 113-124, 1958. Prijs no 176 f 0,50.
- No 177. HOOF, H. A. VAN, Some considerations on the transmission of non-persistent viruses by aphids. Proc. 3rd Conf. Potato Virus Diseases: 114-116, 1958. Prijs no 177 f 0,25.
- No 178. EVENHUIS, H. H., Investigations on a leafhopper-borne clover virus. Proc. 3rd Conf. Potato Virus Diseases: 251-254, 1958. Prijs no 178 en 179 samen f 0,25.
- No 179. EVENHUIS, H. H., De vectoren van het bloemvergroeningsvirus van klaver. (The vectors of the virus causing phyllody (virescence) in clover flowers) T. Pl.-ziekten, 64: 335-336, 1958. Prijs no 178 en 179 samen f 0,25.
- No 180. BEEMSTER, A. B. R., Some aspects of mature plant resistance to viruses in the potato. Proc. 3rd Conf. Potato Virus Diseases: 212-217, 1958. Prijs no 180 f 0,35.
- No 181. EVENHUIS, H. H., Over de invloed van de winter op de parasitering van de appelbloed-luis, *Eriosoma lanigerum*, door haar parasiet *Aphelinus mali*. (The influence of the winter on the parasitism of the woolly aphid, *Eriosoma lanigerum*, by its parasite *Aphelinus mali*) T. Pl.-ziekten, 64: 328-332, 1958. Prijs no 181 f 0,30.
- No 182. BOS, L. en J. P. H. VAN DER WANT, Virusziekten van vlinderbloemigen. (Virus diseases of leguminous plants) Landbouwwoorlichting, 15: 550-558 en 573-587, 1958. Prijs no 182 f 1,15.
- No 183. Franssen, C. J. H. en P. Huisman, De levenswijze en bestrijdingsmogelijkheden van de vroege akkertrips (*Thrips angusticeps* Uzel. (The biology and control of *Thrips angusticeps* Uzel) Versl. landbouwk. Onderz., 64. 10: 1-104, 1958. Prijs no 183 f 3,50
- No 184. Fluiter, H. J. de, Bladluisbestrijding ter voorkoming van virusverspreiding in aard-beien. (Aphid control in strawberries to prevent spread of strawberry viruses) Meded. Landbouwhoges. Gent, 23:745-769, 1958. Prijs no 184 f 0,95